

In re application of
Matthew Patricelli
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Atty. Dkt. No. 063391-0202

Amendments to the Claims/Listing of Claims

Please amend claims 1, 10 and 37, cancel claims 5, 11, 12 and 14-36, and add new claims 38-48 as follows. This listing of claims will replace all prior versions, and listings, of claims in the application:

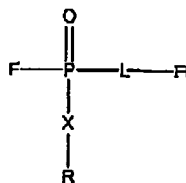
1. (Currently amended) A method for analyzing a complex protein mixture, said method comprising:

labeling one or more active target proteins present in said complex protein mixture by combining at least one probe [~~[-said probe(s) comprising a functional group]~~] specific for one or more active target proteins [~~[[and a fluorescent moiety,]]~~] with said complex protein mixture under conditions whereby said probe(s) covalently react with said active target proteins;

isolating one or more of said labeled active target proteins by binding to a receptor bound to a solid phase, wherein said receptor binds the probe labeling said active target proteins, removing unbound proteins, and releasing bound labeled active target proteins from said receptor; and

detecting a signal from one or more labeled active target proteins present in said complex protein mixture following said isolating, wherein said signal is detected by separating one or more of said labeled active target proteins and generating a fluorescent signal from one or more of said labeled active target proteins during or following said separation;

wherein said probe has the structure:



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wherein:

X is $-\text{CH}_2-$, $-\text{O}-$, or $-\text{S}-$;

R is $-\text{H}$ or a chain of from 1-20 carbon atoms and from 0 to 5 heteroatoms, which chain is straight or branched alkyl, alkenyl, or alkynyl optionally comprising one or more aromatic, alicyclic, heteroaromatic, or heterocyclic groups;

L is a linker moiety comprising from about 2 to 20 carbon atoms and having from 0 to 10 heteroatoms, wherein L is aliphatic, alicyclic, aromatic or heterocyclic; and

Fl is a fluorescent moiety.

2. (Original) A method according to Claim 1, wherein said separation comprises applying all or a portion of said labeled active target proteins to an electrophoretic medium for separation of said labeled active target proteins; and

generating a fluorescent signal from one or more separated active target proteins, whereby said fluorescent signal indicates the presence of an active target protein in said complex protein mixture reactive with said at least one probe.

3. (Original) A method according to Claim 2, wherein said separation comprises SDS-PAGE.

4. (Original) A method according to Claim 2, wherein said separation comprises capillary electrophoresis.

5. (Cancelled)

6. (Original) A method according to Claim 2, further comprising:
isolating at least one fluorescent band from said electrophoretic medium; and
identifying one or more labeled active target proteins present in said fluorescent band.

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7. (Cancelled)
8. (Previously presented) A method according to Claim 1, wherein said fluorescent moiety exhibits a peak absorbance wavelength in the visible spectrum, and exhibits a peak emission wavelength in the visible spectrum.
9. (Original) A method according to Claim 1, wherein said fluorescent moiety is a rhodamine.
10. (Currently amended) A method according to Claim ~~[[7]]~~ 9, wherein said rhodamine is 5-carboxytetramethylrhodamine or 6- carboxytetramethylrhodamine.
- 11-12. (Cancelled)
13. (Original) A method according to Claim 1, wherein said complex protein mixture is a proteome.
- 14-36. (Cancelled)
37. (Currently amended) ~~[[The]]~~ A method ~~[[of]]~~ according to claim 1, wherein said receptor comprises an antibody which binds said probe.
38. (New) A method for analyzing a complex protein mixture, said method comprising:
labeling one or more active target proteins present in said complex protein mixture by combining at least one probe specific for one or more active target proteins with said complex

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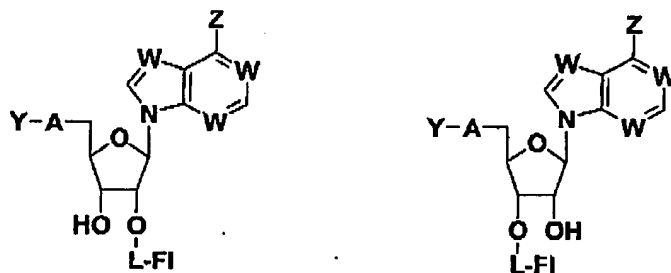
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protein mixture under conditions whereby said probe(s) covalently react with said active target proteins;

isolating one or more of said labeled active target proteins by binding to a receptor bound to a solid phase, wherein said receptor binds the probe labeling said active target proteins, removing unbound proteins, and releasing bound labeled active target proteins from said receptor; and

detecting a signal from one or more labeled active target proteins present in said complex protein mixture following said isolating, wherein said signal is detected by separating one or more of said labeled active target proteins and generating a fluorescent signal from one or more of said labeled active target proteins during or following said separation;

wherein said probe has the structure:



wherein:

each W is independently carbon or nitrogen;

Z is hydrogen or amino;

Y is a functional group capable of reacting with at least one of thiol, hydroxyl or amino joined through A to the 5' carbon of the ribose, where the functional group may be directly bonded to A or through a link, the functional group being one or more moieties comprising halogen, O, S, N, P, or C, selected from the group consisting of fluorosulfonyl, fluorophosphonyl ester, halogen, epoxide, ethylene α to an activating group, and halogen β to an activating group;

A is NR, O, S or CH₂, wherein R is H or alkyl of from 1 to 6 carbon atoms; and

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F1 is a fluorescent moiety joined to the oxygen of the 2' or 3' position of the ribose through a linker moiety L of at least 2 atoms, said linker moiety L comprising carbon, oxygen, nitrogen or sulfur.

39. (New) A method according to Claim 38, wherein said separation comprises applying all or a portion of said labeled active target proteins to an electrophoretic medium for separation of said labeled active target proteins; and

generating a fluorescent signal from one or more separated active target proteins, whereby said fluorescent signal indicates the presence of an active target protein in said complex protein mixture reactive with said at least one probe.

40. (New) A method according to Claim 39, wherein said separation comprises SDS-PAGE.

41. (New) A method according to Claim 39, wherein said separation comprises capillary electrophoresis.

42. (New) A method according to Claim 38, wherein said functional group is selected from the group consisting of an alkylating functionality, an acylating functionality, a ketone functionality, an epoxide functionality, an aldehyde functionality, a sulphonyl functionality and a phosphoryl functionality.

43. (New) A method according to Claim 39, further comprising:
isolating at least one fluorescent band from said electrophoretic medium; and
identifying one or more labeled active target proteins present in said fluorescent band.

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44. (New) A method according to Claim 38, wherein said fluorescent moiety exhibits a peak absorbance wavelength in the visible spectrum, and exhibits a peak emission wavelength in the visible spectrum.

45. (New) A method according to Claim 38, wherein said fluorescent moiety is a rhodamine.

46. (New) A method according to Claim 45, wherein said rhodamine is 5-carboxytetramethylrhodamine or 6-carboxytetramethylrhodamine.

47. (New) A method according to Claim 38, wherein said complex protein mixture is a proteome.

48. (New) A method according to Claim 38, wherein said receptor comprises an antibody which binds said probe.